

Applicant : Bruce A. Morgan
Serial No. : 10/037,667
Filed : October 25, 2001
Page : 4

Attorney's Docket No.: 10287-044001 / MGH 1287.1

REMARKS

Applicant hereby submits that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and amend sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050, referencing attorney docket number 10287-044001.

Respectfully submitted,

Date: 18 July 2002

Leda Tiominis, Reg. No. 50,635
for: Louis Myers
Reg. No. 35,965

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

“Version With Markings to Show Changes Made”

In the specification:

Paragraph beginning at page 15, line 1, has been amended as follows:

Figure 1B depicts the predicted amino acid sequence of Daedalos (Daed; SEQ ID NO:[2] 1), aligned with the other Ikaros gene family members, Helios (Hel; SEQ ID NO:[6] 2), Aiolos (Aio; SEQ ID NO:[8] 3), and Ikaros (Ik; SEQ ID NO:[10] 4). Residues conserved in Ikaros family members are highlighted in gray and the zinc finger domains are boxed.

Paragraph beginning at page 15, line 5, has been amended as follows:

Figure 1C depicts the amino acid sequence of the Xenopus Daedalos (xDaed; SEQ ID NO:[4] 5) protein, aligned with the amino acid sequence of the mouse Daedalos (mDaed) protein (SEQ ID NO:[2] 1).

Paragraph beginning at page 16, line 15, has been amended as follows:

A fourth member of the Ikaros gene family, designated Daedalos, was cloned using PCR with degenerate primers (Morgan et al. (1997) EMBO J 16:2004; Honma et al. (1999) FEBS Letters 447:76). PCR amplification was performed as follows. 40 cycles (95°, 30 seconds; 45°, 1.5 minutes; 72°, 2 minutes) were carried out in a Pfu buffer containing 3 mM MgSO₄, using degenerate primers designed from conserved regions of the murine Ikaros family of proteins: DEG 10 (TG (T/C)AA(T/C)CA(A/G)TG(T/C)GGIGCI (T/A)CITT(T/C)AC; SEQ ID NO:[11] 6) and DEG 12 (TG(G/A)CAICCCAT(G/A)TGIATIGT (G/A)(T/A)ACAT; SEQ ID NO:[12] 7). This resulted in the amplification of a 900 base pair product. 3' and 5' RACE (Marathon, Promega) were employed to clone the remaining coding sequences for each transcript as well as the 5' and 3' UTRs.

Paragraph beginning at page 19, line 3, has been amended as follows:

PCR analysis of Daedalus transcripts confirmed that they are expressed from stage 11 while primary neurogenesis is occurring. Total RNA was prepared from 100 *Xenopus laevis* embryos at stage 11 or 12 and 2 micrograms were reverse transcribed. 165 nanograms of cDNA products (16.5 ng for histone H-4) were amplified in the presence of 1.5 μ Ci each of [P32] dATP and [P32] dCTP using the following primer pairs: histone H-4 (20 cycles, using primers 5'-AGGGACAACATCCAGGGCATCACC (SEQ ID NO:[13] 8) and 3'-ATCCATGGCGGTAACGGTCTTCCT (SEQ ID NO:[14] 9)); XDaedalus (31 cycles, using primers 5'-ATTCTGTAACACTACGCTTGTCGTCG (SEQ ID NO:[15] 10) and 3'-AACAATIGCCATAAGCAGTGTCCA (SEQ ID NO:[16] 11)); and neurogenin-1b (28 cycles, using primers 5'-CATATTGGTACAGGACTCCTATCC (SEQ ID NO:[17] 12) and 3'-CTTGACCCTTATGGGAAGCAGGAA (SEQ ID NO:[18] 13)). The number of cycles employed were in the range for linear amplification of each target. The products were separated on a 5% polyacrylamide gel and quantitated on a phosphorimager (Molecular Dynamics). Input cDNA levels were corrected to achieve similar histone H-4 content.